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(RESEARCH ARTICLE)

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Mycological analysis in three schools over four seasons using passive air sedimentation

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Abstract

The aim of the study is the isolation and identification of fungi using passive air sedimentation. This study analyzed 540 mycological samples from three primary schools in Zenica, collected in September, December, February, and May. Each season, 135 samples were taken from five rooms (two classrooms, a gym, a locker room, and a library) in each school. Samples were collected three times daily at three different heights with 15-minute exposure times. Samples were refrigerated and transported in sterile bags, incubated for 24 hours, and inoculated on specific agars with and without additives. Plates were incubated at 37°C and 25°C for up to 7 days, followed by examinations. Petri dishes were used for passive air sampling, and colonies were counted after incubation. The average number of microorganisms (CFU/m^3) was calculated using Omeliansky's method. Statistical methods included the Chi-squared test and p-value. Colony appearance was assessed visually and microscopically using a light microscope. Growth rate, size, structure, and color changes were monitored. In September, the highest mold concentrations were at H. Kikić Primary School (796 CFU/m^3, not significant), M. Dizdar Primary School (1260 CFU/m^3, not significant), and A. Šantić Primary School (3980 CFU/m^3, significant). *Penicillium* spp. and *Alternaria* spp. were most prevalent, with *Alternaria* spp. significant at H. Kikić Primary School. In December, the highest mold/yeast concentrations were at H. Kikić Primary School (4578 CFU/m^3, not significant), M. Dizdar Primary School (1924 CFU/m^3, significant), and A. Šantić Primary School (2587 CFU/m^3, not significant). *Penicillium* spp. was most prevalent. In February, the highest mold concentrations were at H. Kikić Primary School (4578 CFU/m^3, not significant), M. Dizdar Primary School (2786 CFU/m^3, not significant), and A. Šantić Primary School (5838 CFU/m^3, significant). *Aspergillus* spp. and *Penicillium* spp. were equally prevalent. In May, the highest mold/yeast concentrations were at H. Kikić Primary School (6568 CFU/m^3, significant), M. Dizdar Primary School (3516 CFU/m^3, significant), and A. Šantić Primary School (7431 CFU/m^3, significant). *Aspergillus* spp. was most prevalent. These findings highlight the importance of regular monitoring and implementing appropriate ventilation measures to manage air quality and health concerns in schools.

Keywords: Fungal load; Taxonomic diversity; Elementary education; Seasonal fluctuations; Passive air sampling

1. Introduction

Fungi present in the air within school rooms can have a major influence on the health and well-being of both students and staff. These microorganisms thrive in environments that are damp, dark, and poorly ventilated, allowing them to multiply rapidly. High humidity levels and insufficient ventilation create ideal conditions for fungal growth, which can lead to a variety of health issues, including allergies, asthma, and other respiratory ailments [3,10,11,4,16]. One of the primary sources of moisture in school rooms is condensation, which occurs due to temperature differences between the indoor and outdoor environments, particularly during the winter months. Additionally, water leaks from pipes or roofs can further exacerbate the problem. Fungi can manifest on walls, floors, ceilings, and within ventilation systems, often accompanied by an unpleasant odor and visible stains [3,7,12]. To mitigate fungal growth, it is essential to

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maintain an optimal humidity level, ideally between 40% and 60%. Regular ventilation of rooms, the use of dehumidifiers, and ensuring proper thermal insulation can significantly reduce the risk of fungal proliferation. Furthermore, routine cleaning and maintenance of ventilation systems are crucial in preventing the spread of fungal spores [2,18]. If fungi have already established themselves, it is necessary to take measures to remove them, including the use of specialized cleaning and disinfection agents. In more severe cases, professional remediation services may be required to thoroughly address the issue [5,15,17]. Maintaining healthy and clean air in school rooms is vital for creating a safe and comfortable learning environment, thereby protecting the health of everyone who occupies these spaces. Ensuring proper air quality can enhance concentration, reduce absenteeism due to illness, and promote overall well-being [1,8,9].

2. Material and methods

2.1. Location and Sampling Period

The study was conducted in three primary schools located in the administrative area of Zenica. Sampling took place in September, December, February, and May.

2.2. Number of Samples and Rooms Included in the Study

In total, 540 samples were analyzed for mycological content, with 135 samples collected each season (45 samples per school). In each school, the research included five rooms: two classrooms, a gym, a locker room, and a school library.

2.3. Sampling Schedule and Method

Samples were collected three times a day: in the morning before classes began, during classes, and after classes ended. In each room examined, nine air samples were taken by positioning Petri dishes at three different heights, with an exposure time of 15 minutes.

2.4. Transport, Incubation, and Isolation of Fungi

After sampling, the samples were transferred to a portable refrigerator (from 1°C to 8°C) and transported to the laboratory, where they were incubated for 24 hours. For the isolation of molds and yeasts, samples were inoculated on Sabouraud Maltose Agar (SMA) and Sabouraud Dextrose Agar (SDA), (for subculturing) with and without chloramphenicol and with actidione. Incubation and Examination of Samples: One group of inoculated plates was incubated aerobically for up to 7 days at 37°C, and the other group at 25°C, after which macroscopic and microscopic examinations were performed.

2.5. Passive Air Sampling Method

Petri dishes were used for passive air sampling (BAS ISO 10600-17), where molds and yeasts settled under the action of gravitational force on an open surface. The number of microorganisms in the air was determined by simply counting the colonies that grew on the nutrient media after incubation. The average value of the number of microorganisms CFU/m^3 of air was calculated according to Omeliansky [14]. Formula for Calculating the Number of Fungi in the Air: The total number of fungi in a cubic meter of air is calculated using the formula: Number of molds/yeasts in a cubic meter of air = 10 000 x N / (S x K).

N - number of colonies on the agar surface; S - inner surface of the Petri dish (50.24 cm^2); K - constant for 15 minutes of exposure (K=3).

2.6. Statistical Methods and Colony Assessment

The statistical methods applied in the results include the Chi-squared test (χ^2 test) and the p-value.
Visual and Microscopic Assessment of Colonies: During subculturing, the appearance of colonies was assessed visually, macroscopically, while the microscopic examination of stained specimens was conducted using a light microscope (Olympus Cx41).

3. Results and discussion

3.1. Mold Concentrations in September

In September, passive air sampling at SMA showed the highest concentrations of mold (Figure 1) were:

a) At H. Kikić Primary School, 12 (796 CFU/m³), sampled in the locker room, at a height of 1 m, after classes had ended, but this is not statistically significant (χ2=14.48635; p=0.56253);
b) At M. Dizdar Primary School, 19 (1260 CFU/m³), sampled in the gym, at a height of 2 m, during classes, but this is not statistically significant (χ2=333203; p=0.996972);
c) At A. Šantić Primary School, 60 (3980 CFU/m³), sampled in the gym, at a height of 1 m, after classes had ended, which is statistically significant (χ2=16.6537; p=0.033923).

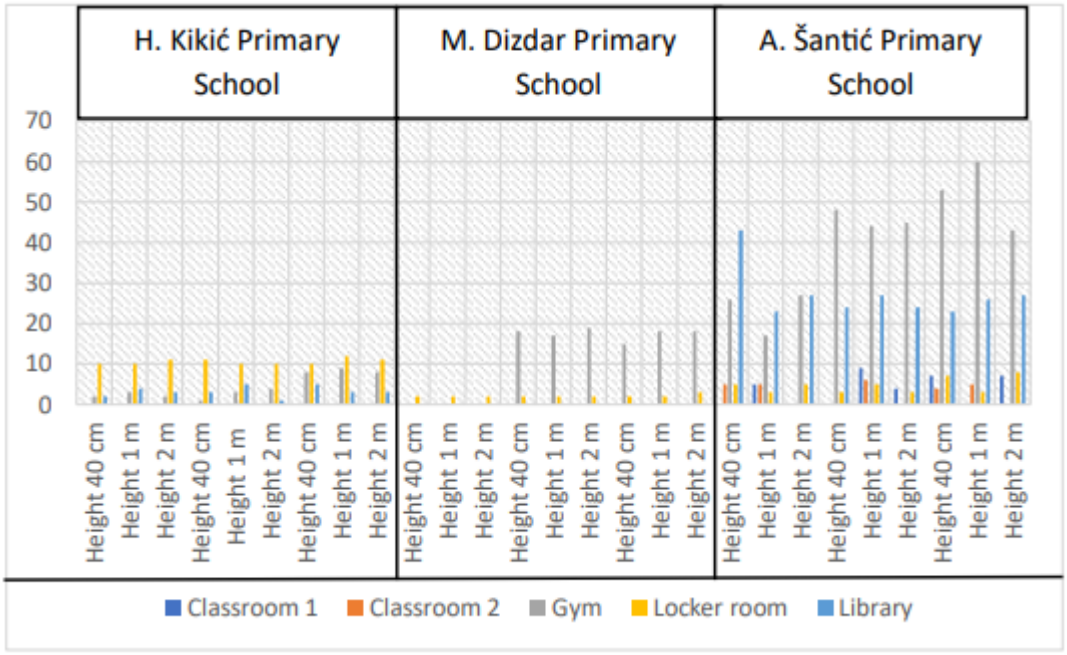

Figure 1 Mycological air analysis – SMA (passive air sedimentation)

When observed in September, the mold isolates from passive air sedimentation showed that *Penicillium* spp. and *Alternaria* spp. were the most prevalent, with *Alternaria* spp. being particularly significant at H. Kikić Primary School, which is statistically highly significant (χ2=13.69668; p=0.008329); (Figure 2).

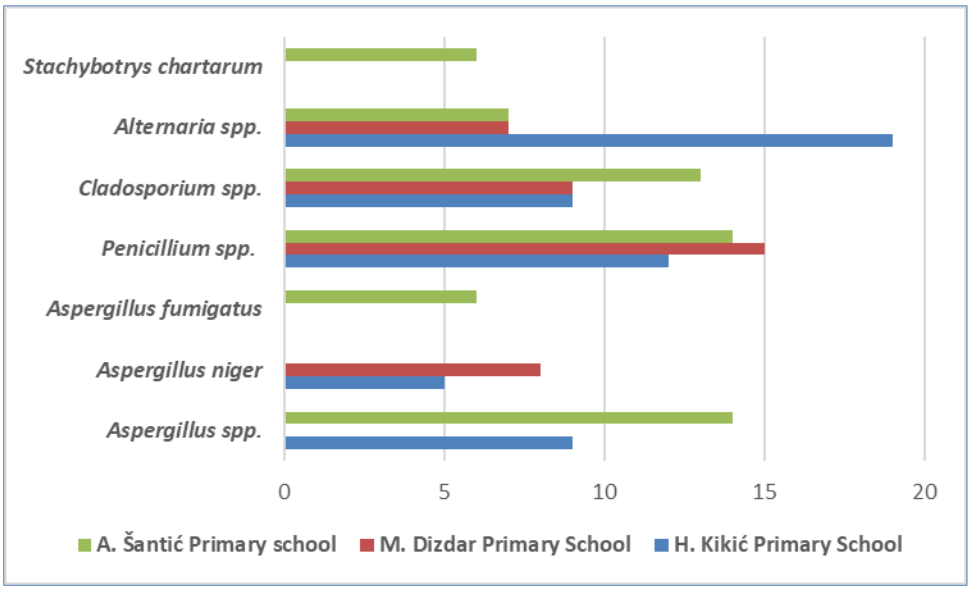

Figure 2 Type and number of mold isolates by passive air sedimentation**.**

In Figure 3 we have an example of *Stachybotris chartarum* on the ceiling and wall in the primary school A. Šantić.

Figure 3 *Stachybotris chartarum* on the ceiling and wall in A. Šantić Primary School**.**

3.2. Mold Concentrations in December

In December, passive air sampling at SMA showed the highest concentrations of mold (Figure 4) were:

a) At H. Kikić Primary School, 69 (4578 CFU/m3), sampled in the locker room, at a height of 1 m, during classes, but this is not statistically significant ($\chi 2=1.817159$; $p=0.98611$);
b) At M. Dizdar Primary School, 29 (1924 CFU/m3), sampled in the gym, at a height of 1 m, after classes had ended and in the locker room at a height of 40 cm after classes had ended, which is statistically significant ($\chi 2=18.31324$; $p=0.018997$);
c) At A. Šantić Primary School, 39 (2587 CFU/m3), sampled in the library, at a height of 2 m, after classes had ended, but this is not statistically significant ($\chi 2=18.98991$; $p=0.966661$).

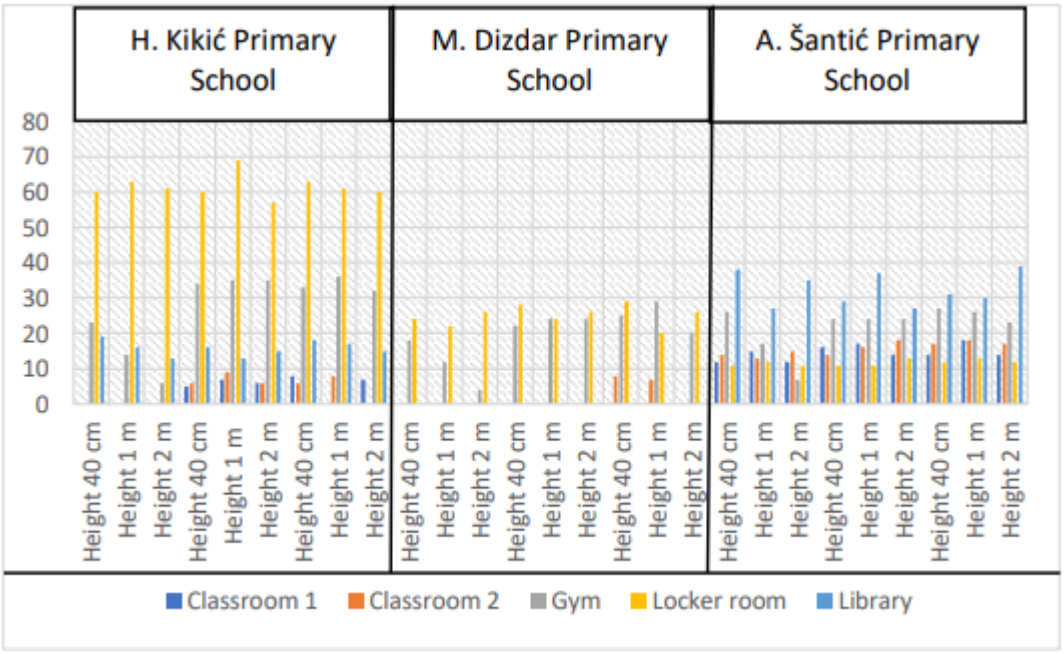

Figure 4 Mycological air analysis – SMA (passive air sedimentation)

When observed in December, the mold/yeast isolates from passive air sedimentation showed that *Penicillium* spp. was the most prevalent compared to other molds. It was most prevalent at A. Šantić Primary School, but this is not statistically significant ($\chi 2=0.200706$; $p=0.904518$); (Figure 5).

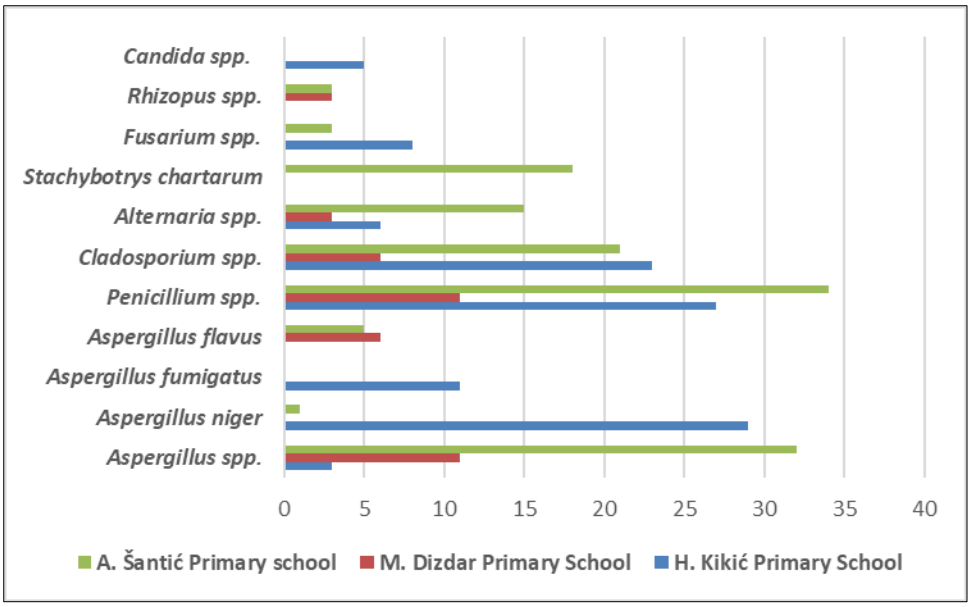

Figure 5 Type and number of mold and yeast isolates by passive air sedimentation

Figure 6 *Aspergillus niger* at A. Šantić Primary School.

3.3. Mold Concentrations in February

In February, passive air sampling at SMA revealed the highest concentrations of mold (Figure 7) were:

- At H. Kikić Primary School, 69 (4578 CFU/m3), sampled in the locker room, at a height of 1 m, after classes had ended, but this is not statistically significant ($\chi 2=1.055647$; $p=0.997872$);
- At M. Dizdar Primary School, 42 (2786 CFU/m3), sampled in the gym, at a height of 2 m, after classes had ended, but this is not statistically significant ($\chi 2=2.742288$; $p=0.949475$);
- At A. Šantić Primary School, 88 (5838 CFU/m3), sampled in the library, at a height of 1 m, before classes started and during classes, which is highly statistically significant ($\chi 2=109.6246$; $p<0.001$).

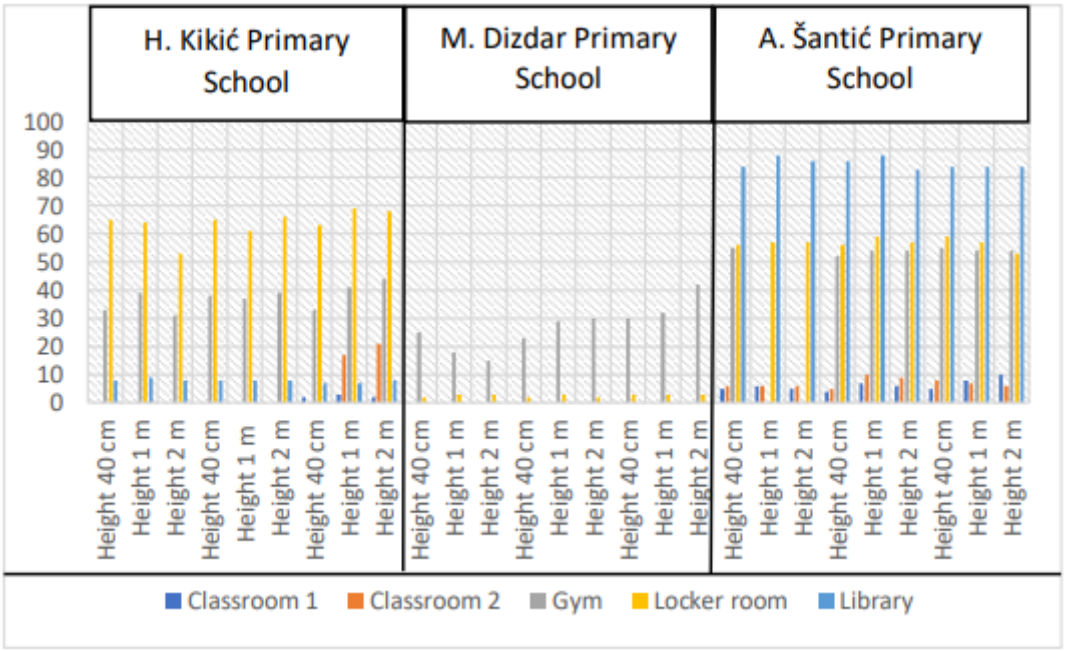

Figure 7 Mycological air analysis – SMA (passive air sedimentation)

When observed in February, the mold isolates from passive air sedimentation showed that *Aspergillus* spp. at A. Šantić Primary School and *Penicillium* spp. at H. Kikić Primary School were equally prevalent compared to other molds, but this is not statistically significant ($\chi 2=1.075709$; $p=0.898107$); (Figure 8).

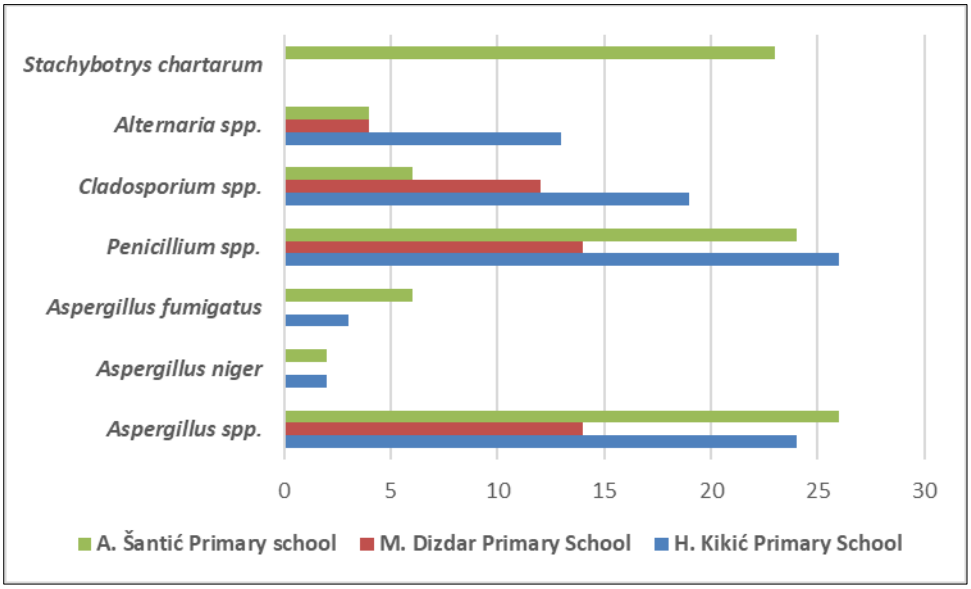

Figure 8 Type and number of mold isolates by passive air sedimentation

3.4. Mold Concentrations in May

In May, passive air sampling at SMA indicated the highest concentrations of mold/yeast (Figure 9) were:

- At H. Kikić Primary School, 99 (6568 CFU/m3), sampled in the gym, at a height of 40 cm, during classes, which is highly statistically significant ($\chi 2=71.21933$; $p<0.001$);
- At M. Dizdar Primary School, 53 (3516 CFU/m3), sampled in the locker room, at a height of 40 cm, after classes had ended, which is highly statistically significant ($\chi 2=27.13003$; $p=0.000671$);
- At A. Šantić Primary School, 112 (7431 CFU/m3), sampled in classroom 2, at a height of 1 m, before classes started, which is highly statistically significant ($\chi 2=442.2105$; $p<0.001$).

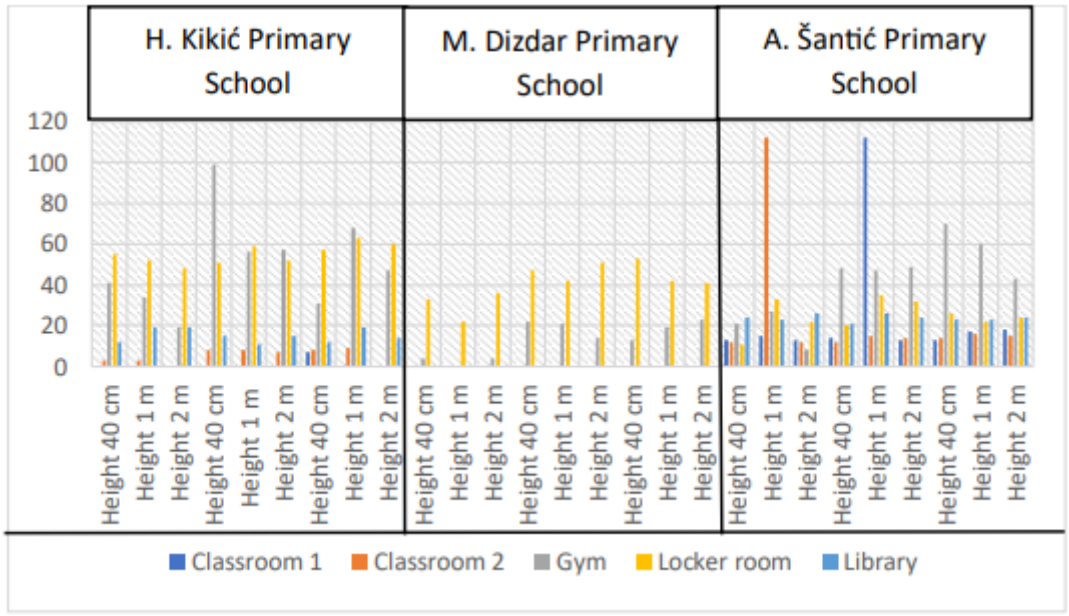

Figure 9 Mycological air analysis – SMA (passive air sedimentation)

When observed in May, the mold/yeast isolates from passive air sedimentation showed that *Aspergillus* spp. was the most prevalent at A. Šantić Primary School, but this is not statistically significant ($\chi 2=0.501606$; $p=0.778176$); (Figure 10).

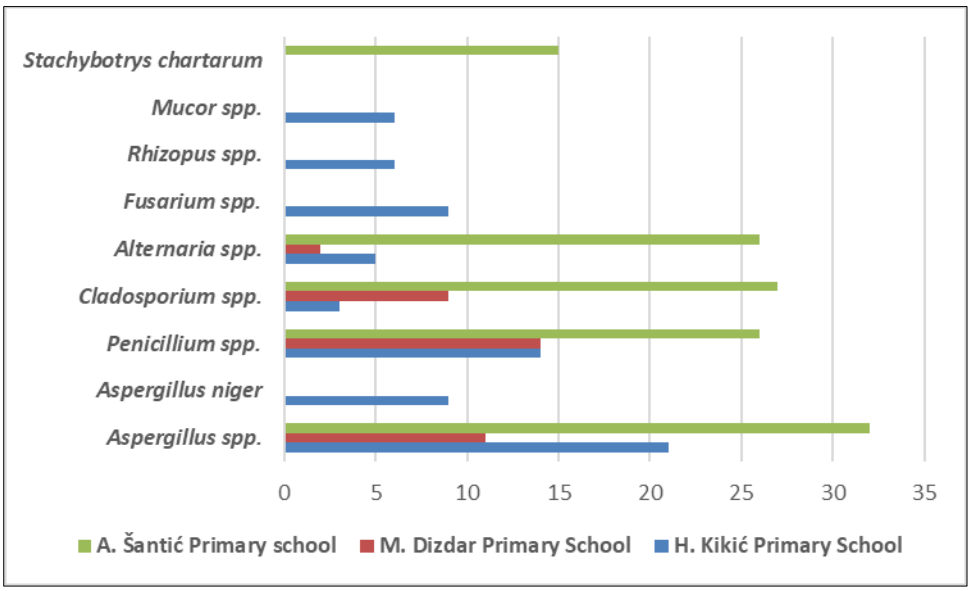

Figure 10 Type and number of mold/yeast isolates by passive air sedimentation

In Figure 11, we have an example of *Aspergillus niger* on a part of the ventilation system in the locker room at H. Kikić Primary School.

Figure 11 *Aspergillus niger* on a part of the ventilation system in the locker room at H. Kikić Primary School

Regular ventilation helps maintain air quality in classrooms and other rooms because fresh outdoor air contains more oxygen and fewer pollutants compared to indoor air. Good air quality helps students stay focused and productive, while poor air quality with excess carbon dioxide can cause fatigue, drowsiness, and difficulty learning. Additionally, fresh air helps remove microorganisms and improves overall health. Ventilating rooms contributes to a more pleasant and fresher environment for students and teachers [19,20,21].

In two out of three schools examined, certain problems with unventilated school spaces were noticeable. It is especially important to highlight the result of the isolation and identification of *Stachybotrys chartarum* (Figure 3) in the classrooms of the A. Šantić primary school. The mentioned isolate is visible in larger accumulations on the classroom walls. A high level of humidity can negatively affect the air quality and the health of those present. The architectural solution of the locker rooms in the newly built H. Kikić primary school has insufficient ventilation capacity, as shown by the results of the isolation of the mold *Aspergillus niger* (Figure 11), which is present on the surfaces of the ventilation system. The locker rooms are without windows, and the ventilation system only works when the light is on.

Considering that classrooms are spaces where students spend a significant part of the day, it is important to ensure optimal air humidity for their comfort and concentration. High humidity can lead to the formation of fungi on walls and ceilings, which can be harmful to human health, especially for individuals with respiratory issues. The presence of fungi on walls and ceilings over a long period can lead to the destruction of building materials. Locker rooms in sports halls are areas where moisture often accumulates, especially after showering and sweating during sports activities. Such an environment can be ideal for the development of fungi and bacteria, so it is important to maintain good ventilation and regular cleaning. The library is a room where a large amount of books and other paper materials are often stored, which are sensitive to moisture. High levels of humidity can cause damage to books, such as changing the shape of the paper, the appearance of stains and mold, which can reduce their value and lifespan. In other school areas, such as corridors, offices, or physical education halls, it is also important to control the level of humidity to maintain a healthy and safe working environment for all present. Regular room ventilation, the use of air humidifiers, repairs of damage to walls and windows, as well as the maintenance of heating and cooling systems can help regulate moisture and prevent potential problems. In cold weather, excessive condensation and freezing on windows, and sometimes on exposed walls, cause the relative humidity to drop below the recommended values of 40-60% [22,23,24,25]. In the city school M. Dizdar, due to urban heating before the start of classes in the first shift, air humidity values of 16% were recorded in classroom 2.

In the three examined schools sampled for four seasons, the highest fungi concentrations were found in A. Šantić primary school at a height of 1 meter before the start of classes: in May, in classroom 2 (Figure 9). The result was 112 (7431 CFU/m^3).

In September, based on microbiological testing of 153 isolates obtained by passive air sedimentation in three examined schools, the most prevalent was ***Alternaria* spp.** (n=19; 26%) originating from H. Kikić primary school (Figure 2). In December, out of 284 isolates, the most prevalent was the ***Penicillium* spp.** (n=34; 26%) originating from A. Šantić primary school (Figure 5). In February, out of 229 isolates, the most prevalent were the ***Penicillium* spp.** (n=26; 30%) from H. Kikić primary school and the ***Aspergillus* spp.** (n=26; 27%) from A. Šantić primary school (Figure 8). In May, out of 235 isolates, the most prevalent was the ***Aspergillus* spp.** (n=32; 25%) from A. Šantić primary school (Figure 10). In a study conducted in two schools in Poland after the heating season ended, out of 321 samples, the number of isolates amounted to 379. Passive air sedimentation sampling found that the most commonly represented mold genera were: *Aspergillus*, *Penicillium*, and *Cladosporium* (6). By comparing our study with this one, we obtained similar results.

In our own research conducted in May, the results were (Figure 10): ***Aspergillus* spp.** (64/235), ***Penicillium*** spp. (54/235), and ***Cladosporium*** spp. (27/235).

Moisture control is key to preserving health and the quality of buildings. Prolonged moisture can cause fungi, metal corrosion, swelling and rotting of wooden components, and damage to paints and varnishes. Studies show that in 70% of cases of building problems, the direct or indirect cause is indeed moisture [26,27].

4. Conclusion

The study conducted through passive air sedimentation on Sabouraud Maltose Agar across three schools over four seasons revealed significant findings regarding mold concentrations and types. The highest mold concentration was recorded in A. Šantić primary school in May, with a notable 7431 CFU/m^3 in classroom 2 before the start of classes. Throughout the seasons, different mold genera were predominant: in September, *Alternaria* spp. was the most prevalent (26%) at H. Kikić primary school; in December, *Penicillium* spp. dominated (26%) at A. Šantić primary school; in February, *Penicillium* spp. (30%) at H. Kikić primary school and *Aspergillus* spp. (27%) at A. Šantić primary school were the most common; and in May, *Aspergillus* spp. was the most prevalent (25%) at A. Šantić primary school. These findings highlight the importance of regular monitoring and implementing appropriate ventilation measures to manage air quality and health concerns. Ensuring a healthy and safe environment for students and staff should be a priority, with regular maintenance, proper cleaning, and hygiene education being key strategies to reduce mold concentrations and protect the health of all school occupants.

Compliance with ethical standards

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Disclosure of Conflict of interest

The authors declare no conflict of interest.

Statement of informed consent

The research was approved by the school management.

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